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INACTIVATION OF BACILLUS SUBTILIS SPORES BY ULTRAVIOLET AND SOLUX LIGHTING

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by

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1. **anthrax**
2. **solux light**
3. **bacillus subtilis**

Abstract

An intentional outbreak of anthrax (*Bacillus anthracis*) created a scare that forced an increasing awareness of this bacterial pathogen. This MQP used a bacterium of the same genus, *Bacillus subtilis*, to determine whether the use of ultraviolet light and simulated sunlight (Solux) would be able to inactivate the spores after various lengths of time, ranging from two minutes to forty-eight hours. The results showed that both sources of light were successful in the inactivation of *B. subtilis* spores.

Acknowledgement

I would like to thank Professor Theodore C. Crusberg for his guidance and the use of his lab for the completion of this project.

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Introduction

Bioterrorism is defined as terrorism involving the use of biological warfare agents such as disease-causing organisms or herbicides. The subject of bioterrorism has been an issue ever since the unintentional outbreak of smallpox in the U.K. that occurred many years ago to the outbreak of anthrax that happened just 4 years ago. These two diseases are the most serious hazards in bioterrorism because of their ability to disperse easily. As for anthrax, we know that it cannot be easily spread from human to human, although there is some conjuncture about this and the Black Death of the 14th century. Still, we think of anthrax as a non-transmissible disease from human to human. This research concentrates on the most recent outbreak of anthrax and how the disease can be contained or destroyed by use of two types of light.

Anthrax, also known as *Bacillus anthracis*, was first discovered by Robert Koch in 1876. In his experiment, he "grew the organism in pure culture, demonstrated its ability to form endospores, and produced experimental anthrax by injecting it into animals" (10). Anthrax has had many roles throughout the course of history, which includes: **1880** – First successful immunization of livestock against anthrax, **1943** – United States begins developing anthrax weapons, and **2001** – A letter containing anthrax spores was mailed to NBC one week after the September 11 terrorist attacks on the Pentagon and World Trade Center. The technique of spreading the disease by use of spores through the mail caused a scare in which people were advised to check their mail for suspicious letters and/or packages and post offices were advised to check all mail and implement decontamination methods for sterilizing incoming mail.

Researchers have yet to find an effective way to kill the *B. anthracis* spores other than treating the disease with antibiotics after it has infected an individual. This research was focused on how to inactivate the spores using two light sources. Two different sources of light were used to test whether or not either source would be able to kill the spores: ultraviolet light and a different type of light that is nearly similar to sunlight – Solux light. Because of *B. anthracis* being hazardous to work with, a bacterium similar to anthrax was used. The bacterium *Bacillus subtilis* is from the same genus as anthrax, but is much safer to work with.

B. subtilis was obtained from stock and serial diluted to 3 different numbers of spores: ten thousand, one hundred thousand, and one million. The setup of the experiments were designed, both using a suction apparatus to place the *B. subtilis* spores onto a Millipore membrane filter and placed into a Petri dish containing nutrient agar. Two different sets of time intervals were used for each light source and each set of serial dilutions (10^4, 10^5, 10^6) was used for each time interval set. The experiments determined how much time and what type of light source would be able to kill the spores.

Bacteria

Bacillus anthracis – *B. anthracis* is a large, aerobic or facultative Gram-positive bacterium that is able to form spores. The common name for this bacterium that causes this disease is Anthrax, which caused a total of 22 cases within the U.S. in 2001 by contamination from U.S. mail. This bacterium is very similar to both *Bacillus cereus* and *Bacillus thuringiensis*, genotypically and pheotypically. Humans can become infected incidentally

when they are brought into contact with diseased animals such as cattle, sheep, and pigs, which include their flesh, bones, hides, hair, and excrement. The anthrax spores are able to survive in infected soil and animals for many decades. One example is that if the spores remain dry on filter paper, they can survive for 41 years (1). Transmission from person to person is non-transmissible with anthrax. There are 3 different ways that anthrax can infect a person:

- **Cutaneous anthrax** develops when the bacteria enter your body through superficial cuts or wounds and cause lesions on your skin. The vast majority of anthrax infections are cutaneous.

- **Intestinal anthrax** results from eating food contaminated with the anthrax bacteria. Both cutaneous and intestinal anthrax are not usually lethal.

- **Inhalational anthrax** occurs when you breathe in the anthrax bacterial spores into your lungs. The spores can originate from contaminated soil or from the skins and hides of infected animals. This form of anthrax is usually fatal.

The mechanism of death from the anthrax toxin is still not fully understood at this time. Death from the disease is due to oxygen depletion, secondary shock, respiratory failure, and cardiac failure. There are three components that contribute to the anthrax toxin: 1) the edema factor (EF), 2) the protective antigen (PA), and 3) the lethal factor (LF). Certain combinations of the three factors will either result in an illness or nothing at all.

PA + LF = lethal activity
EF + PA = edema
EF + LF = inactive
PA + LF + EF = edema and necrosis, lethal

A certain strain, which is called the Sterne strain of *B. anthracis*, produces sublethal amounts of the toxin that induces formation of a protective antibody (10).

The symptoms of anthrax resemble the flu, with fever, chills, and muscle aches. For inhalation anthrax, the symptoms resemble a respiratory illness and would progress to severe shortness of breath and a low concentration of oxygen in the blood (hypoxia). In order to treat anthrax, the disease has to be identified as the cause of the disease and be treated with antibiotics such as ciprofloxacin (Cipro).

Bacillus subtilis – *B. subtilis* is a close relative to *Bacillus anthracis*, but it is not nearly as lethal. It has been linked to food borne illnesses that cause nausea, diarrhea, or vomiting. Because it can tolerate a wide range of environmental stresses, *B. subtilis* produces a spore coat that surrounds the cortex of the spore. This coat allows *B. subtilis* to enter into a dormant state and produce spores in a process called sporulation. Functions of the coat include serving as a barrier against entry of large toxic molecules and participating in germination (2). The coat consists of two major layers: a lightly staining inner coat and a darkly staining outer coat. Researchers have been arguing whether or not there exists a layer outside the outer coat of the spore called the exosporium. This layer is absent in most *Bacillus* species and relates more to the accommodation to specific lifestyles rather than germination. The exosporium imparts other abilities that researchers have probably not discovered yet, as well as not being readily detected by light microscopy (2). It also possesses proteins that are able to resist against environmental factors.

The reason why *B. subtilis* spores are able to resist environmental changes and remain in a dormant state before and after exposure to sunlight was because it was thought that “they were suited for quantitative studies on the genetic effects caused by sunlight”

(6). The process of sporulation begins when the environment in which the *Bacillus* cells thrive in becomes harsh. When the environment changes, the *Bacillus* cells begin to produce spores in response to the change, creating a resistant endospore. For *Bacillus subtilis*, the cell can perform sporulation in about eight hours. The endospores can "remain dormant for many years, but when conditions allow, they can convert back to vegetative cells fairly rapidly" (12). This process is known as germination. After exposure to UV light, the mechanisms in the spore begin to correct and repair DNA damage, which result in the spores becoming less resistant and resuming normal cell function after the cell breaks out of its spore coat.

Dipicolinic acid, which plays a role in the survival of the *B. subtilis* spores when exposed to artificial and solar UV radiation, enhances the spore survival to solar UV (8). Spores with decreased levels of DPA are more resistant to UV irradiation at 254 nm and DPA increases the yield of the thymine-thymine dimer (8). This effect of DPA may explain the high resistance of vegetative cells, which lacks DPA, to 254 nm irradiation. It is this element that aids *Bacillus subtilis* in resistance against ultraviolet light. It has also been mentioned that *B. anthracis* spores are three to four more times resistant to 254 nm UV light than *B. subtilis* spores (9). In order to completely inactivate *B. anthracis* spores, it would require an amount of approximately 46,000 lux of UV light. For *B. subtilis* spores, an amount of 22,000 lux of UV light would be sufficient enough (11).

Figure 1: Diagram of a Bacterial Spore (http://gsbs.utmb.edu/microbook/ch015.htm)

Figure 2: Process of Sporulation and Germination of a Bacterial Spore
(http://www.arches.uga.edu/~howie/formation.html)

Light Sources

Two light sources were used in this study. One light source was a laboratory ultraviolet light box used for DNA and RNA identification in gels, and the other was an incandescent source usually employed to illuminate museum specimens.

Ultraviolet Light – UV light is divided into three regions: UV-A, UV-B, and UV-C. The UV-A region ranges from 400 to 320 nm and is the low energy UV; the UV-B region is from 320 to 290 nm and is the harmful region of the UV spectrum; and the UV-C region ranges from 290 to 100 nm and is absorbed by the Earth's atmosphere. The UV box that was used for this experiment (Spectroline Transilluminator, TX-302) has a wavelength of 302 nm, which was able to do harm to the *B. subtilis* spores.

Solux Light – The manufacturer's specifications of the Solux bulbs that were used in this study were from Eiko (Eiko, Ltd., Shawnee, Kansas 66227) (http://www.eiko-ltd.com/main.asp) indicate that these bulbs have a color temperature of 4700 Kelvin, wattage of 50 W, and a voltage from 12 – 16 V. The degree of the beam is 10º, which means that the light beam is very narrow over a certain surface area. This type of light is used mainly in museums because of its similarity to sunlight. The color rendering index, or CRI, of natural sunlight is 100. Solux bulbs have a CRI between 98 and 99, which is extremely close to that of daylight (5). Figure 3 shows the similarity of the Solux 4700 and the daylight 4700. The use of this lamp at 16 V provides a good approximation for natural

sunlight. Bulb life at 12 V is perhaps 1000 hours, but at 16 V, it is only in the range of 3 to 4 days.

Figure 3: Solux vs. Daylight (http://www.wiko.com/specgraphs.html)

Materials and Methods

UVT Plexiglass – This glass, also known as polymethylmethacrylate, has a thickness of 1/8 in. and is photolytically resistant to ultraviolet light, but allows transmission of UV light from wavelengths between 280 and 360 nm. Conventional plexiglass contains additives which totally block UV light. It does have poor resistance to low temperature, fatigue, and solvents. It was used to determine at what wavelength the Solux light would be able to penetrate through the glass. From Figure 4, ultraviolet light at a wavelength of at least 325 nm will have a high transmittance percentage through UVT.

Figure 4: UVT Plexiglass Transmittance under Various Wavelengths (http://www.atoglas.com)

Flexible thin film UV filter – A flexible UV absorbing film (Flexible Thin Film UV Filter, Cat. No. NT39-426) was obtained from Edmund Optics (Barrington, NJ). The type of film used in this experiment was a flexible thin film UV filter. The film has a thickness of 0.04 mm and is specially coated to absorb ultraviolet rays. This filter has a transmission of less than 10% if the wavelength of the UV light is below 390 nm (3). Figure 5 displays the transmittance of the UV film.

Figure 5: UV Film Transmittance under Various Wavelengths
(http://www.edmundoptics.com/onlinecatalog/displayproduct.cfm?productID=1873)

Preparation of membrane filter and Petri Dishes – In order to prepare the number of spores from serial dilutions, the original stock of *B. subtilis* spores had to be diluted to one million spores. The original concentration of stock, provided by Prof. Crusberg, was 3.56 x 10^8; the desired number of spores was 1 x 10^6; the desired volume was 10 mL of one million spores; the unknown is the volume of stock to use. The calculations for the serial dilutions are as follows:

$C_1V_1 = C_2V_2$

$(3.56 \times 10^8)\ V_1 = (1 \times 10^6)\ (10\ mL)$

V_1 = 28 µL stock + 9.972 mL dH_2O = 10^6 spores/mL (1,000,000)

Serial dilutions

10^5 spores/mL = 1 mL of 10^6 spores + 9 mL dH_2O (100,000)

10^4 spores/mL = 1 mL of 10^5 spores + 9 mL dH_2O (10,000)

In summation, in order to obtain one million spores, 28 µL of stock and 9.972 mL dH_2O was transferred into a 15 mL sterile conical tube. To obtain the other dilutions, 1 mL of the previous stock was micropipetted into 9 mL dH_2O in a 15 mL sterile conical tube. Once the dilutions were complete, the spores were placed in a Petri dish. In order to do so, they were captured on a 0.22-µm Millipore (Billerica, MA, GNWP02500) membrane filter. A suction apparatus was used to place the spores onto the filter, which is shown in Figure 6.

Figure 6: Suction Apparatus

The base and the funnel in the apparatus (both are combined by the blue clamp) were flame sterilized with 70% ethanol before and after placing the spores onto the filter. The base was first sterilized with ethanol and then by flame. The filter was then removed from its sterile package by using sterile tweezers and was placed on the base. The glass funnel was placed onto the base and then clamped down. Once it was set up, 5 mL of dH_2O and 1 mL of 10^6 stock was added to the funnel and settled for about a minute, and then was suctioned out through into Erlenmeyer flask. The clamp was released and the funnel was removed, leaving the filter on the base. The filter was removed from the base with sterile tweezers, placed in a Petri dish, and then covered to prevent contamination. The process was repeated for 10^4 and 10^5 spores.

Ultraviolet Light Irradiation – With the Petri dishes prepared, they are ready to be irradiated by ultraviolet light. The wavelength of the Spectroline transilluminator UV box that was used is approximately 302 nm, which is located in the harmful portion of the UV spectrum. The image of the UV box is shown in Figure 7.

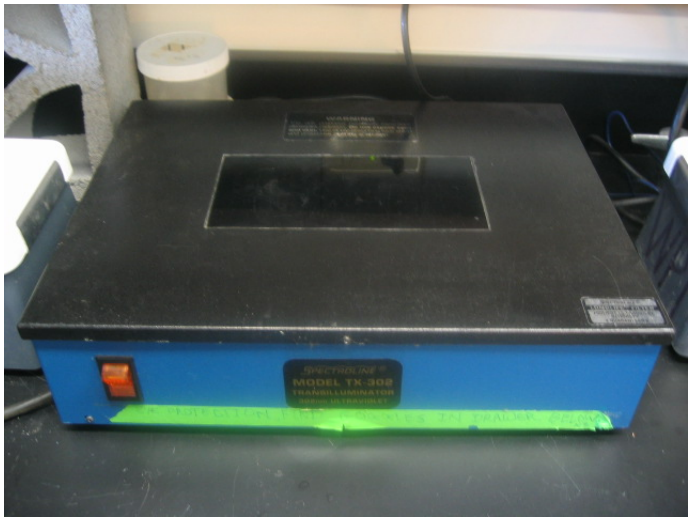

Figure 7: Spectroline Transilluminator 302 nm UV box

The glass area in the center of the UV box was where the Petri dishes were placed upon. Three Petri dishes, each with a different dilution, were placed on the glass upside-down, with the covers off, so that the spores on the filters received full exposure of the UV light. While the UV box was switched on, the lights in the room were turned off to prevent further exposure. This experiment was done in time intervals of 2, 5, 10, 30, and 60 minutes, for a total of 15 Petri dishes used. Once illumination to each set of Petri dishes was completed in their respective time interval, the covers were put back onto the Petri dishes, then "covered in aluminium foil to prevent accidental exposure to sunlight" and subsequent photoreactivation (7), and placed in an incubator overnight.

Solux Light Inactivation – The Solux light apparatus is shown in Figure 8.

Figure 8: Solux Light Apparatus and Irradiation

Two strips of 3 Solux bulbs were lined up with a Petri dish directly underneath each light in the beam, which had a beam angle of 10 degrees for each lamp. Each Petri dish had a different number of spores (10^4, 10^5, 10^6) on Millipore filters, respectively. On a regular sunny day, the number of lux of sunlight is around 100,000. The amount of lux on the spores at 12 V was around 42,000 and at 16 V, it was around 83,000 lux. Again, the covers were removed, but this time, they were covered with a UV-transmitting plexiglass disk that allowed the UV-rays from the Solux bulbs to penetrate through the glass, yet protected the filters from airborne microbial contaminants. The Petri dish plastic (polystyrene) absorbs UV light, thereby requiring substitution of the UVT plastic. This experiment was carried out twice but under different voltages: 12 V and 16 V. One setback was that the maximum voltage of a Solux bulb was 16 V and if the light setup was plugged directly into a wall outlet, all 3 bulbs would blow because the outlet has an output

of 120 V. To set the bulbs to these two voltages, the setup itself was plugged into a Variac to lower the voltage to 16 V. Two Variacs were used; one was an older model and one was a newer model. Figure 9 shows the newer Variac that was used.

Figure 9: Newer Variac Model

The exposure times for this experiment were 12, 24, 36, and 48 hours, using a total of 30 Petri dishes. The room lights were not a factor in this experiment since the spores were exposed to the light during the daytime and night time. Similar to the UV experiment, the Petri dishes were re-covered, wrapped in aluminium foil, and placed in the incubator overnight.

The type of light meter that was used for this experiment was the EXTECH EasyView 30 Light Meter (EXTECH Instruments, Model EA30, http://www.extechproducts.com/). This particular light meter can measure up to 400,000 lux and 40,000 foot candles. Figure 10 shows the light meter used in the lab.

Figure 10: EXTECH EA 30 Light Meter
(http://www.testequipmentdepot.com/extech/lightmeters/ea30.htm)

Results

UV light experiment

After overnight incubation, the Petri dishes were ready for analysis. The main goal was to determine how much time it would take for the UV light to fully inactivate the *B. subtilis* spores. For the least amount of UV exposure, more colonies survived. As the time increased, less colonies grew or none at all. Table 1 shows the results of the UV experiment.

Time Exposure	# of Spores	# of Colonies
2 min	1000000	1000
2 min	100000	600
2 min	10000	200
5 min	1000000	200
5 min	100000	100
5 min	10000	40
15 min	1000000	12
15 min	100000	2
15 min	10000	0
30 min	1000000	0
30 min	100000	0
30 min	10000	0
60 min	1000000	0
60 min	100000	0
60 min	10000	0

Table 1: UV Light Exposure Results

At 2, 5, and 15 minutes, still some colony growth was observed, but at 30 and 60 minutes, there was absolutely no growth i.e. complete spore inactivation was achieved. Figure 11 shows the same data and graphically shows the decrease in colonies over time.

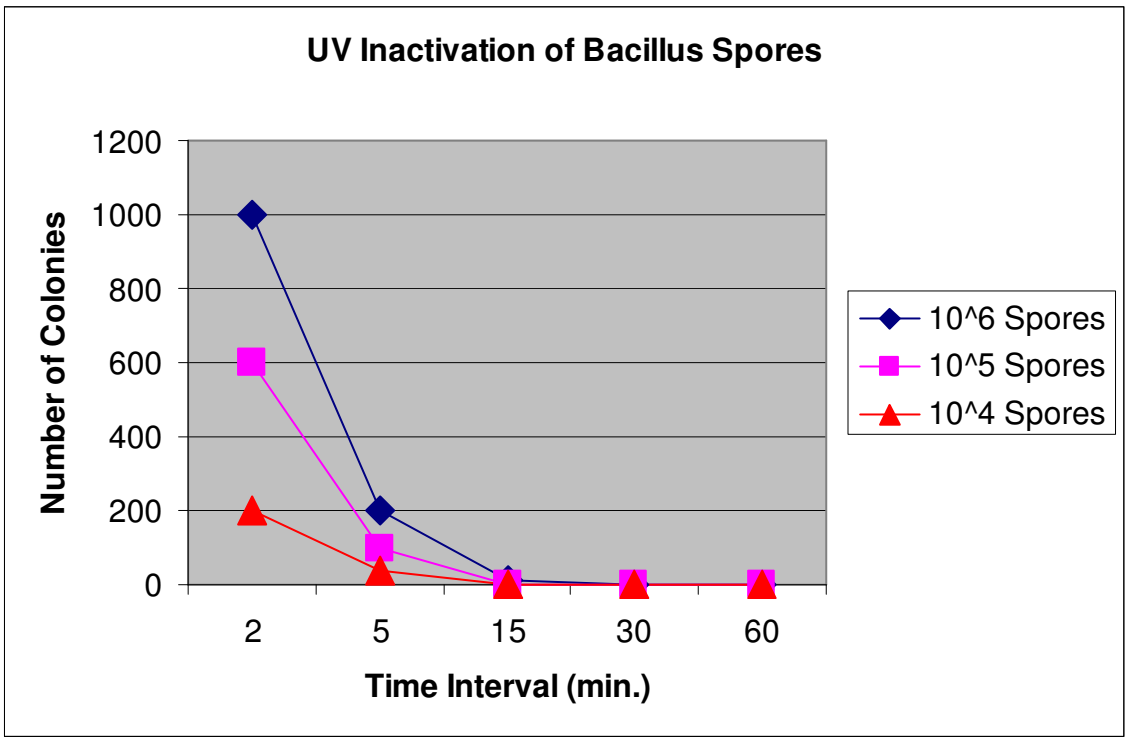

Figure 11: Decreased Growth of Colonies over Time due to UV Irradiation

In this figure, the points were for a single determination; each point represented a single Petri dish at a specific time. One observation from Figure 10 was that for each number of spores, the number of colonies decreased significantly from 2 to 5 minutes. The ideal time to completely inactivate these spores was 15 minutes; 20 minutes to be exact.

Solux light experiment

There are two different sets of results due to the use of two different voltages: 12 V and 16 V. Both sets of results are nearly identical with the exception of the 12 V data showing colony growth after 48 hours while the 16 V showed no growth after 48 hours. Table 2 shows the results for the 12 V experiment and Table 3 shows the results for the 16 V experiment.

Time Exposure	# of Spores	# of Colonies
12 hrs	1000000	1500
12 hrs	100000	1200
12 hrs	10000	400
24 hrs	1000000	1200
24 hrs	100000	750
24 hrs	10000	250
36 hrs	1000000	400
36 hrs	100000	300
36 hrs	10000	175
48 hrs	1000000	200
48 hrs	100000	150
48 hrs	10000	100

Table 2: Solux Light Exposure at 12 V Results

Time Exposure	# of Spores	# of Colonies
12 hrs	1000000	1200
12 hrs	100000	700
12 hrs	10000	350
24 hrs	1000000	500
24 hrs	100000	300
24 hrs	10000	200
36 hrs	1000000	50
36 hrs	100000	3
36 hrs	10000	1
48 hrs	1000000	0
48 hrs	100000	0
48 hrs	10000	0

Table 3: Solux Light Exposure at 16 V Results

The graphs for each set of data show a decrease in the number of colonies after consecutive 12-hour intervals. It shows that after the equivalent of 48 hours of sunlight at 83,000 lux, all of the spores were no longer viable. The amount of lux at 16 V in comparison with the amount of lux of sunlight implicates that the survival of spores depends on how much light they are exposed to. Both graphs are nearly similar to the UV light results (Figure 11). Figures 12 and 13 displays these results.

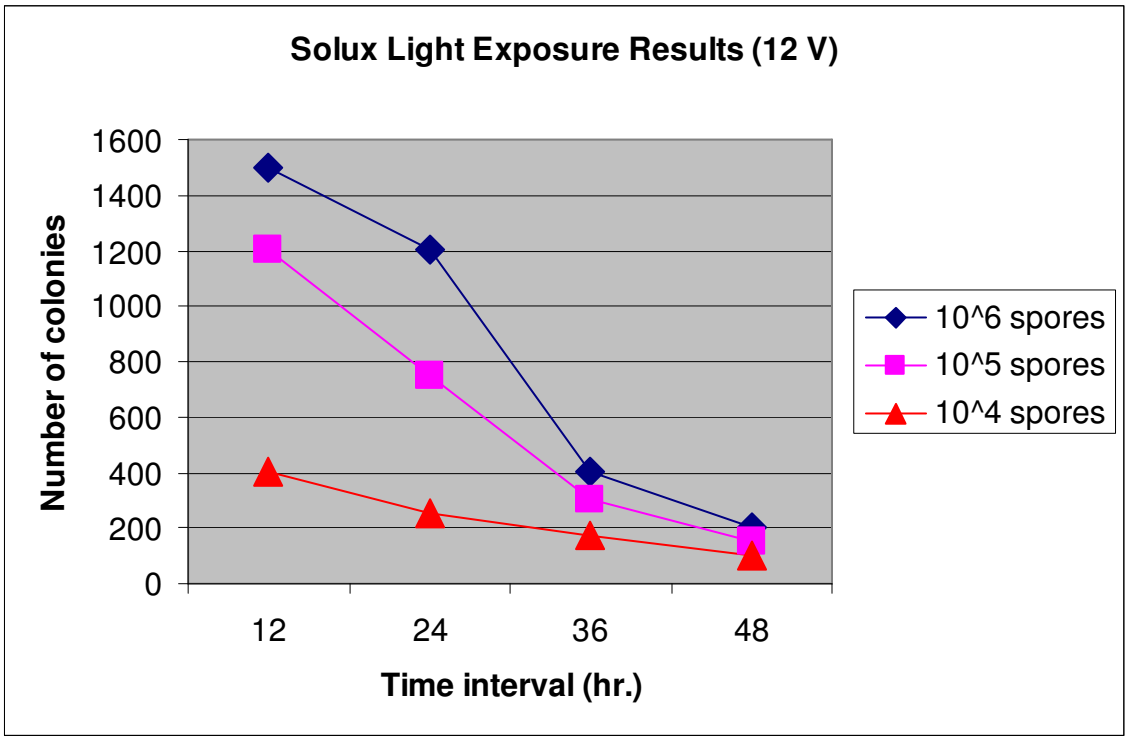

Figure 12: Decreased Number of Colonies from Solux Light at 12 V (per Petri dish)

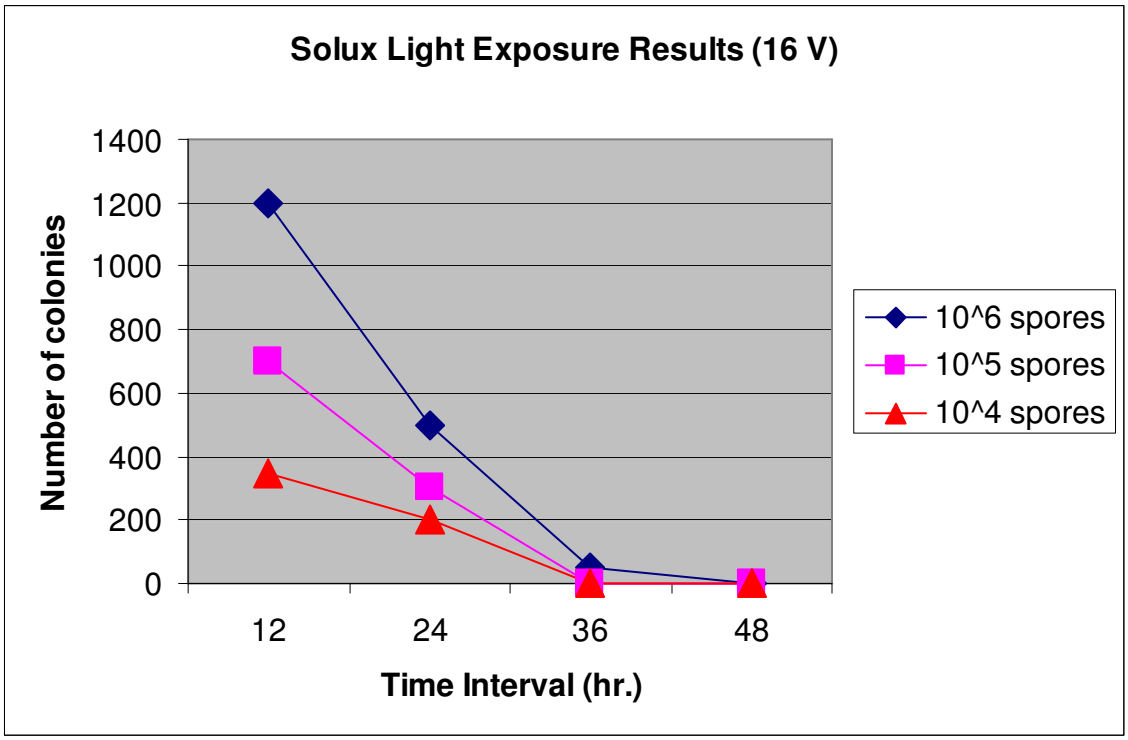

Figure 13: Decreased Number of Colonies from Solux Light at 16 V (per Petri dish)

These results demonstrate that the total time to fully inactivate the *B. subtilis* spores by use of Solux light was 48 hours. To confirm that these results were agreeable with the data and determine any statistical errors, the 48-hour experiment was repeated, but with different setups. The voltage was set at 16 V and the number of spores was set at one million throughout the experiment. Two sets of 3 Petri dishes were used; both sets were covered with the UVT plexiglass, but one set was covered with the UV filter, which was mentioned in the Materials and Methods section, and set directly on top of the UVT glass. Table 4 shows the results of this set of experiments.

Time Exposure	Voltage	# of Spores	# of colonies	Cover Type
48 hrs.	16 V	1000000	30	UVT glass
48 hrs.	16 V	1000000	20	UVT glass
48 hrs.	16 V	1000000	10	UVT glass
48 hrs.	16 V	1000000	TNTC	UV film
48 hrs.	16 V	1000000	TNTC	UV film
48 hrs.	16 V	1000000	TNTC	UV film

Table 4: Confirmation of Successful Data Results

The letters “TNTC” are defined as “too numerous to count”, meaning that the number of colonies that grew on the plate could not be counted by the naked eye. Even after 48 hours, there is still some growth of the colonies, but that result was expected. As stated in the Materials and Methods section, the UV film has a transmittance of less than 10% if the wavelength is less than 390 nm and the UVT glass has a high transmittance if the wavelength is at least 325 nm. The Solux light was unable to penetrate through the UV film but successfully went through the UVT glass. In order to comply with these results, the region of the Solux light would have to be from 325 to 390 nm, which is approximately equal to the UV-A region of the UV spectrum; it is a low energy UV light.

Because these results may not be statistically valid, it would be wise to find the standard deviation, a statistical measure of spread and viability, of each set of Petri dishes and find the range where the colonies lie upon. Tables 5, 6, and 7 shows the standard deviations of each set of data.

Time (min)	# of colonies	StdDev
2	600	± 400
5	113.33	± 80.83
15	4.67	± 6.43
30	0	± 0
60	0	± 0

Table 5: Standard Deviation of UV Results

Time (hr)	# of colonies	StdDev
12	1033.33	± 568.62
24	733.33	± 475.22
36	291.67	± 112.73
48	150	± 50

Table 6: Standard Deviation of Solux Light Results (12 V)

Time (hr)	# of colonies	StdDev
12	750	± 427.2
24	333.33	± 152.75
36	18	± 27.73
48	0	± 0

Table 7: Standard Deviation of Solux Light Results (16 V)

Figures 14, 15, and 16 shows the range of the colonies at each data point. As the time interval increases, the standard deviation becomes smaller until it reaches 0.

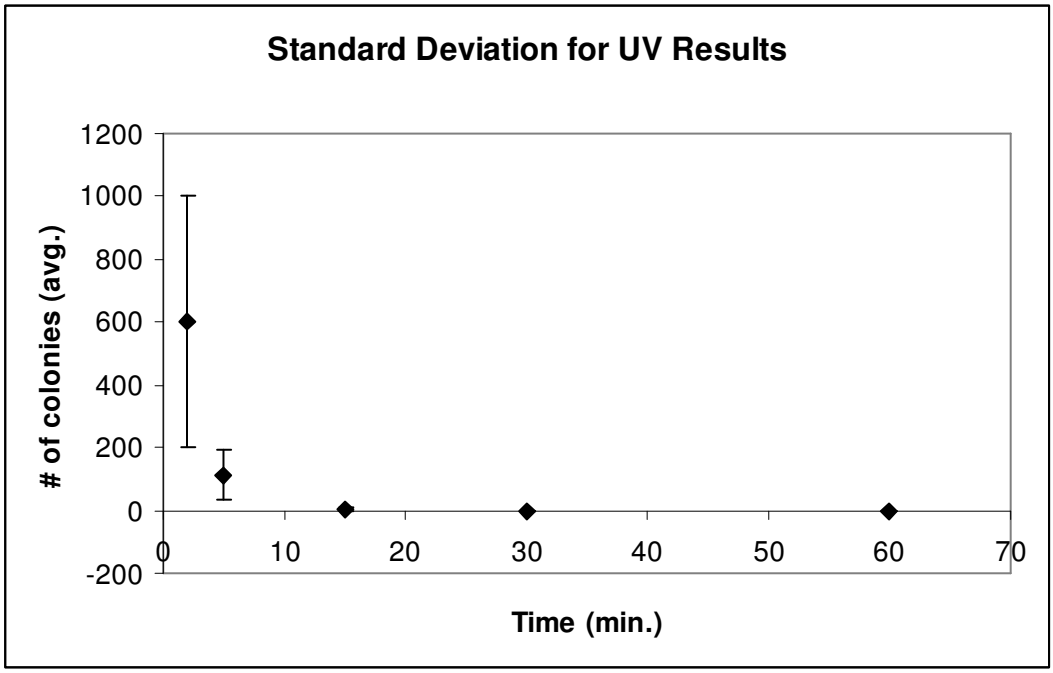

Figure 14: Graph of UV Standard Deviation

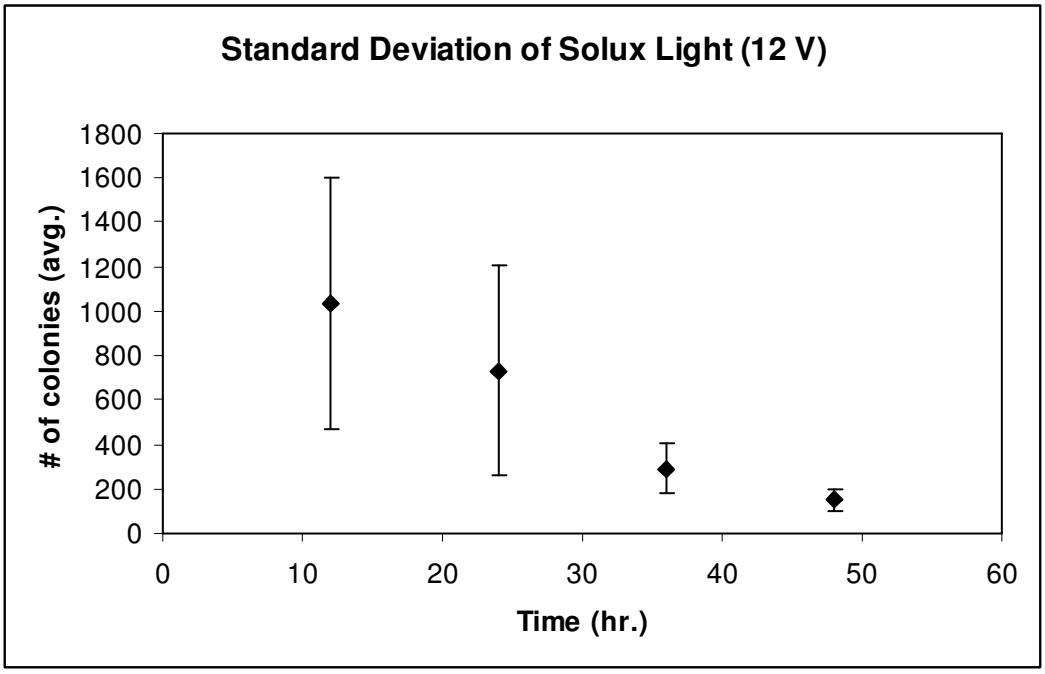

Figure 15: Graph of 12 V Solux Light Standard Deviation

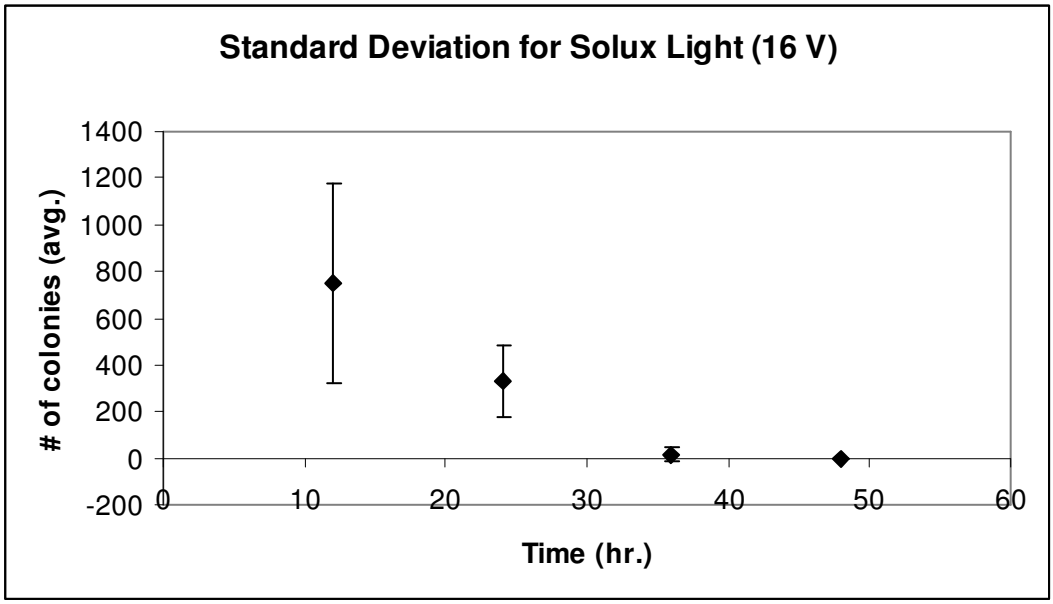

Figure 16: Graph of 16 V Solux Light Standard Deviation

By finding the logarithm of the number of spores, we can insert a least-squares fit line to "minimize the sum of the squares of the distances of the points from the curve" (13). Figures 17, 18, and 19 show the plots of the logarithms of the number of spores, along with the equations of the Least Squares-fit line for each plot. Some of the values are left out because when you take the log of 0, it is equal to $-\infty$, which cannot be plotted on the graphs.

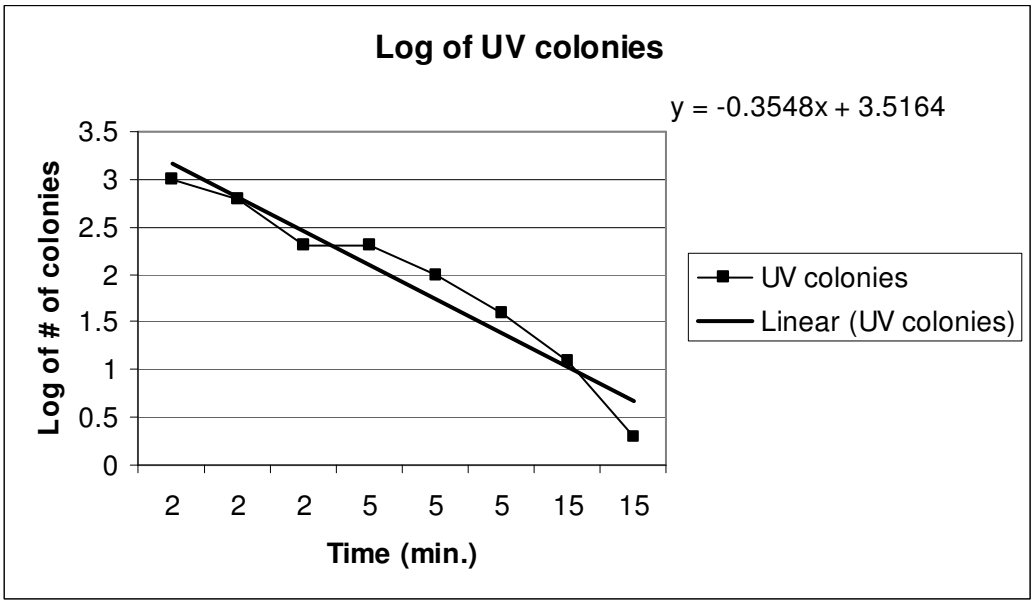

Figure 17: Log Values of UV Colonies

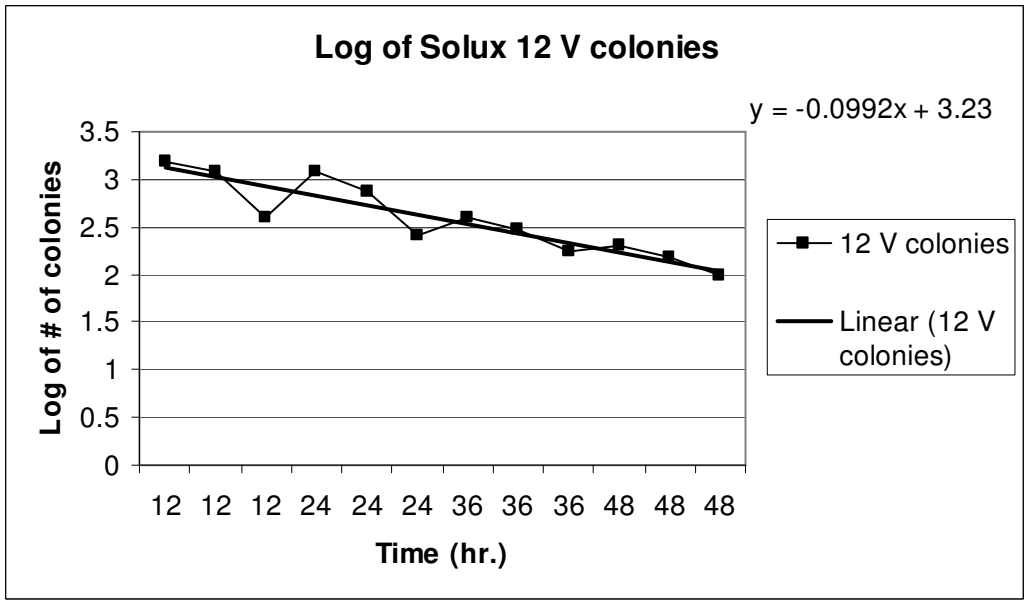

Figure 18: Log Values of Solux Colonies at 12 V

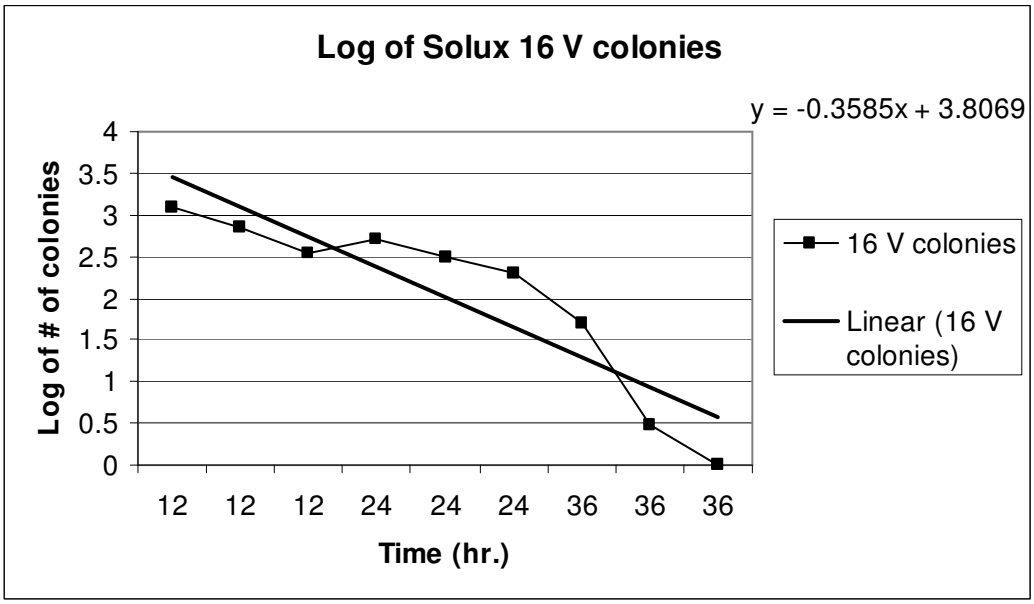

Figure 19: Log Values of Solux Colonies at 16 V

Discussion

Research has been done for many years on the mechanism of ultraviolet light inactivation of *Bacillus subtilis* spores. With UV and Solux light being nearly similar to each other, the Solux light containing a good deal of the UV component of natural sunlight, the results prove that both sources of light were successful in inactivating the *B. subtilis* spores. The UV light took less time than the Solux due to the fact that the wavelength of the UV light was 302 nm, which lies in the UV-B region of the spectrum and is the harmful portion of UV light. By interpreting Figures 4 and 5, the wavelength of the Solux light lies between 325 and 390 nm, which lies in the UV-C region of the spectrum. It takes longer for the Solux light to inactivate the spores because of the fact it is

the low energy UV. For UV, the minimum time it takes to fully inactivate the spores is approximately 20 minutes and for the Solux light, it takes about 48 hours.

Using the 302 nm UV box, Table 1 shows that even after 2 minutes of UV exposure, the number of colonies decreased greatly by about 1000 times the original amount. As more time elapsed, more colonies were inactivated until there was nothing viable left. It probably was not required to exposure the spores for 60 minutes, but it helps if the original data had any errors and weren't sure of the results. Tables 2 and 3 have similar results; both show a decrease in colonies by about 1000 fold after 12 hours of exposure to Solux light. It took the full 48 hours to fully inactivate the spores since Solux light is a low energy UV light. Each dilution of spores (10^4, 10^5, 10^6) maintains the same rate of inactivation at each time interval, as seen in Figures 10, 11, and 12. Each of these figures shows a significant decrease after the second time interval. The data from Table 4 for the Solux light experiment was repeated six times, three times with the UVT plexiglass as the cover and the other three with the thin film UV filter, between 325 and 390 nm. The Solux light was not able to penetrate through the UV filter but was able to fully penetrate the UVT glass. After 48 hours, the results show that there is almost complete inactivation with the UVT glass, but none with the UV filter. Figure 3 shows the transmittance for the UVT plexiglass in which a wavelength of at least 325 nm will have a high transmittance through the glass. Figure 5 shows the transmittance percentage for the UV filter and wavelengths of at least 390 nm will penetrate through the filter at 80% transmittance. The plots for the standard deviations and the least-squares fit lines show that the decrease in the number of colonies is consistent for each time interval.

The purpose of this project was to determine whether or not these two light sources would be able to inactivate bacterial spores. Several experiments have been done before with ultraviolet light and *B. subtilis* spores, but there has not been any research done using Solux light. The ultraviolet experiment has been done already using various techniques by other researchers. The experiments of this project showed that ultraviolet and Solux light are able to inactivate *B. subtilis* spores.

Further experiments beyond these may be to inactivate the spores at different wavelengths, possibly higher or lower than the values used for this project. A study of the protein structure of the *B. subtilis* spores may be useful in determining how these spores are able to resist UV radiation. Perhaps an experiment on how white light can inactivate these spores will also be done in the near future.

The correction and restoration of DNA from within the spore is one of two reasons that make *B. subtilis* and other *Bacillus* spores resistant to UV exposure. The second reason is a spore photoproduct, or SP, that the spores generate during UV irradiation called the thymine-thymine, or T=T, dimer (8). Inactivation of bacteria by ultraviolet light results from the production of cyclobutane-type thymine dimers that form in DNA. The *Bacillus* spores do not generate this type of dimer. Instead, they produce a unique photoproduct: 5-thyminyl-5,6-dihydrothymine (4).

The Solux bulbs were not intended for inactivating spores. The main purpose of these bulbs was to provide simulated artificial sunlight for various environments. But one plausible explanation would be that since Solux light greatly resembles natural sunlight, one would be able to utilize this light for future research on how deadly bacteria are

inactivated naturally by sunlight. This project may be the stepping stone for the beginning of the research using Solux as a solar simulator.

Hopefully, this project will help researchers discover a way to inactivate the deadly *B. anthracis* spores that are still a threat to society. The use of ultraviolet light on humans may be hazardous as well as working on the *B. anthracis* spores themselves. By using Solux, post offices and other areas where anthrax may be a threat will be able to inactivate these spores efficiently without causing harm to the individual. But Solux is not guaranteed to work so further research would have to be done to understand how Solux really works. Both sources of light were able to inactivate the *B. subtilis* spores and may be able to inactivate other species of *Bacillus*. The main goal here is to kill off the *Bacillus anthracis* spores without harming the infected individual in the process and one day, that goal will be reached.

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